PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Donald W. Kufe, *et al.*	Group Art Unit: 1635
Serial No.: 10/778,859	Examiner: Vivlemore, Tracy Ann
Filed: February 13, 2004	Atty. Dkt. No.: DFCI:008US
For: MUC1 Extracellular Domain and Cancer Treatment Compositions and Methods Derived Therefrom	

DECLARATION OF DONALD W. KUFE UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

I, Donald W. Kufe, do declare that:

1. I am a United States citizen residing at 179 Grove St., Wellesley, MA, 02482.

2. I currently hold the position of Professor of Medicine, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, 02115. A copy of my curriculum vitae is attached as Appendix A.

3. I am the first named inventor of the above-referenced U.S. patent application.

4. I have reviewed the Office Action dated July 27, 2006, that is related to the above-referenced application.

5. I understand that the Examiner has rejected claims 1, 2, and 5 as not being enabled by the specification under 35 U.S.C. §112, first paragraph. In particular, I understand that

the Examiner is of the opinion that *in vivo* delivery of interference RNA is unpredictable and the skilled artisan, based on the disclosure of the instant specification, would not know whether *in vivo* administration of interference RNA would result in successful delivery of the RNA to the site of disease with inhibition of a target gene.

6. I disagree with the Examiner's position that the skilled artisan would not know whether *in vivo* administration of interference RNA to a subject would result in successful inhibition of a target gene. My opinion is based on the disclosure of the specification, which demonstrates successful application of siRNA in the inhibition of MUC1 expression *in vitro*, and the state of the art pertaining to RNA interference technology which has demonstrated successful *in vivo* gene inhibition in many arenas, including cancer therapy.

7. The specification of the above-referenced application provides detailed guidance regarding the therapeutic use of interference RNA, including siRNA, in humans. There is detailed information in the specification regarding the structure and function of siRNA, including disclosure of numerous sequences. General guidance regarding therapeutic applications of siRNA can be found, for example, on page 47, line 6 – page 48, line 15. Example 9 describes the effect of siRNA on MUC1 expression in human MCF-breast cancer cells. Specification, page 58, line 10 – page 60, line 12. As shown in FIG. 5, siRNA downregulates MUC1 expression at both the protein level, as shown by the immunoblot results, and the RNA level, as shown by the RT-PCR results. Specification, page 60, lines 8-12 and FIG. 5. FIG. 6 shows the downregulation of MUC1 expression by siRNAs in MCF-7 cells at the protein level in these human cells by immunoblot. Specification, page 60, lines 11-12 and FIG. 6. Example 10 (page 60, line 16 – page 62,

line 10) provides detailed information regarding the combination of MUC1 directed siRNA and cisplatin on human non-small cell lung cancer A549 cells. FIG. 7 shows that MUC1 siRNA transfected A549 cells exhibited an increase in the percentage of apoptotic cells relative to controls and also more than additively enhances the percentage of apoptotic cells when combined with CDDP exposure. Specification, page 62, lines 6-9. FIG. 8 also shows that transfection of two siRNAs decreased the proliferation of A549 cells. Specification, page 62, lines 9-10.

8. Regarding *in vivo* applications of interference RNA, it is noteworthy that numerous studies have demonstrated successful target gene silencing in animal models following local delivery of RNAi. Examples of these studies include the following:

- Bitko *et al.*, Nat. Med. 11:50-55, 2005 (**Exhibit 1**): Inhibition of respiratory viruses by nasally administered siRNA in a mouse model
- Li *et al.*, Nat. Med. 11:944-951, 2005 (**Exhibit 2**): Local siRNA can protect against respiratory viral infections
- Palliser *et al.*, Nature 439:89-94, 2005 (**Exhibit 3**): An siRNA-based microbicide protects mice from lethal herpes simplex virus 2 infection
- Shen *et al.*, Gene Ther. 13:225-234, 2006 (**Exhibit 4**): Suppression of ocular neovascularization with siRNA targeting VEGF receptor in two mouse models
- Reich *et al.*, Mol. Vis. 9:210-216, 2003 (**Exhibit 5**): siRNA targeting VEGF inhibits ocular neovascularization in a mouse model
- Nakamura *et al.*, Mol. Vis. 10:703-711, 2004 (**Exhibit 6**): RNA interference targeting transforming growth factor-beta type II receptor suppresses ocular inflammation and fibrosis

- Thakker *et al.*, Mol. Psychiatry 10:782-789, 2005 (**Exhibit 7**): siRNA-mediated knockdown of the serotonin transporter in the adult mouse brain
- Luo *et al.*, Mol. Pain 1:29, 2005 (**Exhibit 8**): Efficient intrathecal delivery of small interfering RNA to the spinal cord and peripheral neurons
- Makimura *et al.*, BMC Neurosci, 3:18, 2002 (**Exhibit 9**): Reducing hypothalamic AGRP by RNA interference increases metabolic rate and decreases body weight without influencing food intake
- Niu *et al.*, J. Gynecol. Cancer, 16:743-751, 2006 (**Exhibit 10**): Inhibition of HPV 16 EC oncogene expression by RNA interference *in vitro* and *in vivo*.
- Grzelinski *et al.*, Hum Gene Ther. 17:751-766, 2006 (**Exhibit 11**): RNA interference-mediated gene silencing *in vivo* exerts antitumoral effects in glioblastoma xenografts
- Kim *et al.*, Mol. Ther. 14:343-350, 2006 (**Exhibit 12**): Choleseryl oligoarginine delivering VEGF siRNA effectively inhibits tumors growth in colon adenocarcinoma
- Takei *et al.*, Cancer Res. 64:3365-3370, 2004 (**Exhibit 13**): siRNA targeting VEGF as cancer therapeutic.
- Minakuchi *et al.*, Nucleic Acids Res. 32, e109, 2004 (**Exhibit 14**): Atelocollagen-mediated synthetic siRNA delivery for effective gene silencing *in vitro* and *in vivo*.

9. In addition, numerous studies have demonstrated successful target gene silencing in animal models following systemic delivery of RNAi. Examples of these studies include the following:

- Soutschek *et al.*, Nature 432:173-178, 2004 (**Exhibit 15**): Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs in mice

- Zimmerman *et al.*, Nature 441:111-114, 2006 (**Exhibit 16**): RNAi-mediated gene silencing in non-human primates to silence apoB in mice
- Giesbert *et al.*, J. Infect. Dis. 193:1650-1657, 2006 (**Exhibit 17**): Postexposure protection of guinea pigs against a lethal ebola virus challenge is conferred by systemic RNA inference
- Schiffelers *et al.*, Nucleic Acids Res. 32, e149, 2004 (**Exhibit 18**): Cancer siRNA therapy by tumor selective delivery with ligand-targeted sterically stabilized nanoparticles
- Urban-Klein *et al.*, Gene Ther. 12:461-466, 2005 (**Exhibit 19**): RNAi-mediated gene-targeting through systemic application of polyethyleneimine (PEI)-complexed siRNA *in vivo*.

10. Additional information regarding the state of the art pertaining to gene silencing using RNAi therapeutics can be found in the following review articles:
    - Bumcrot *et al.*, Nature Chemical Biology 2(12):711-719, 2006 (**Exhibit 20**): "Therapeutics based on RNA interference (RNAi) offer a powerful method for rapidly identifying specific and potent inhibitors of disease targets from all molecular classes.
    - Dykxhoorn *et al.*, Gene Ther. 13:541-552, 2006 (**Exhibit 21**).
11. The above demonstrates that there have been numerous proof-of-concept studies in animal models of human disease demonstrating the broad application of RNAi in the silencing of gene expression *in vivo*. These studies indicate that *in vivo* administration of interference RNA is feasible, and has not turned out to be a major limitation for the therapeutic use of interference RNA. Therefore, the Examiner's assertion that *in vivo* delivery of interference RNA is a major limitation for the therapeutic use of interference

RNA has not been shown to be the case. The comments cited by the Examiner in Jen *et al.* and Opalinska *et al.* pertaining to limitations of *in vivo* application of RNA interference are merely speculative, and have not been proven to be the case as shown by the foregoing cited references.

12. Therefore, contrary to the assertion of the Examiner, one of ordinary skill in the art would not have been presented with any major limitation in *in vivo* application of the claimed methods. Any experimentation that might be required to practice the claimed invention and to administer the interference RNA in a manner that would result in gene inhibition would be routine to those of ordinary skill in the art, and would certainly not present any undue burden.

13. Further, the Examiner is incorrect in her interpretation that "interference RNA" encompasses "antisense RNA." The term "interference RNA" is well-known in the art, and has a meaning that is distinct from "antisense RNA." As set forth in Bumcrot *et al.* (Exhibit 20), "[i]n RNAi, the target mRNA is enzymatically cleaved, leading to decreased abundance of the corresponding protein." Exhibit 20, page 711. Further, the interference RNA is a "double-stranded RNA." See Exhibit 20, page 711, and FIG. 1. In RNA interference, long double-stranded RNA (dsRNA) is cleaved into small interfering RNA (siRNA). See Exhibit 20, page 712, and FIG. 1. This mechanism is distinct from gene inhibition using antisense RNA, where the antisense RNA is a single-stranded RNA molecule. Thus, "interference RNA" is distinct from "antisense RNA."

14. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so

made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1/22/07
Date

Donald W. Kufe
Donald W. Kufe